Going beyond *Saccharomyces cerevisiae* for the production of bioethanol

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Introduction

In addition to inhibitor tolerance, **pentose fermentation** is a key feature required in any organism used for economically viable bioethanol production with lignocellulosic biomass. Although recent work has succeeded in establishing xylose fermentation in *S. cerevisiae* strains, little is known about the potential of **yeast species other than *S. cerevisiae*** that ferment xylose for bioethanol production.

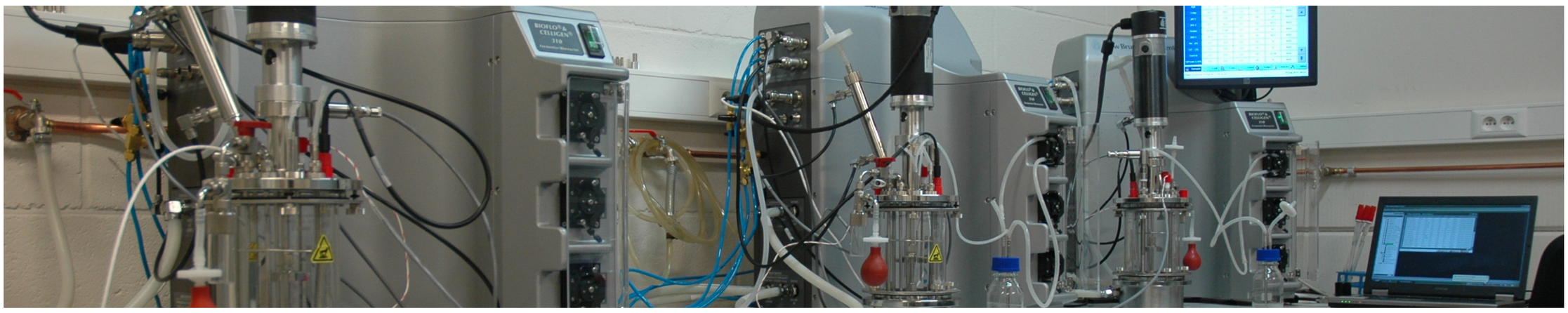

Figure 1: Eppendorf Bioflo 310 fully controlled bioreactors used for bioethanol fermentation experiments

Materials & Methods

A previous screening on solid agar plates of a collection non-Saccharomyces yeasts isolated from sugar-rich environments revealed some strains of different genera that showed promising phenotypes (Table 1). For example, they showed good tolerance to HMF, a major inhibitor in lignocellulosic fermentation. A **selection of strains** was subjected to fermentation experiments under controlled conditions (pH 4.5, 30 ° C, 300 rpm) using a Bioflo 310 bioreactor (Eppendorf, Figure 1). A medium with 7.5% of C6 sugars (6.5% glucose, 0.5% galactose and 0.5% mannose) and 7.5% C5 sugars (7% xylose and 0.5% arabinose) **without and with inhibitors** relevant for lignocellulosic fermentation (acids, furfural, HMF) was used. Results are compared to an industrially used *S. cerevisiae* strain. Samples were taken to measure growth (OD), ethanol, glycerol and sugars.

Results & Conclusions

Fermentation experiments without (-) and with (+) inhibitors were performed with *W. anomalus* (WA) and *S. cerevisiae* (SC) (Figure 2). Only 8% of xylose was consumed by both strains in both experiments. Consumption of xylose was probably due to growth rather than fermentation. Even after complete glucose consumption (within 88h (WA) and 20h (SC)) xylose was not consumed. **Ethanol** concentrations reached resp. **74% and 67%** relative to the initial glucose concentration and 31% and 29% relative to the initial glucose + xylose concentration. Glucose which was not converted to ethanol was probably used for growth during the initial aerobic phase. Ethanol production by SC was similar without and with inhibitors suggesting that the current inhibitor concentrations were not affecting its fermentation. WA reached a similar ethanol concentration, but only after a longer fermentation time. Nevertheless, it reached a higher ethanol yield compared to SC during the fermentation with inhibitors.

Table 1: Selection of potential non-Saccharomyces yeasts of different genera isolated from sugar-rich environments for bioethanol fermentation based on a high throughput screening of aerobic growth on solid agar plates. Values represent growth relative to the control condition (CTRL) (%). *Wickerhamomyces anomalus, Torulaspora delbrueckii* and *Pichia kudriavzevii* showed tolerance up to 10% ethanol. In contrast, *Candida bombi, Starmerella bombicola* and *Metchnikowia spp.* among others showed poor tolerance even at 5% ethanol. HMF tolerance was most pronounced for 1 *C. bombi*, 1 *S. bombicola* and the *P. kudriavzevii*, however, also *W. anomalus* and *T. delbrueckii* showed tolerance up to 4 g/l HMF, a relevant concentration for bioethanol from lignocellulosic material.

ID	Origin	CTRL	Glu 50%	Glu 55%	Glu 60%	Glu 70%	ET 5%	ET 7%	ET 10%	HMF 4g/L	HMF 5g/L	HMF 6g/L	HMF 7g/L
Candida bombi	Nectar	719	42	24	29	22	50	23	0	121	97	78	55
Candida bombi	Nectar	1083	17	9	8	0	0	0	0	0	0	0	0
Hanseniaspora clermontiae	Nectar	1782	10	0	0	0	13	0	0	19	0	0	0
Hanseniasporia uvarum	Nectar	1554	6	0	0	0	19	0	0	15	0	0	0
Starmerella bombicola	Nectar	399	39	26	13	0	30	14	0	93	57	41	0
Starmerella bombicola	Nectar	437	35	26	21	0	14	0	0	73	15	0	0
Metschnikowia pulcherrima	Soil	654	28	34	21	9	10	0	0	170	0	0	0
Metschnikowia aff. Fructicola	Soil	639	44	40	17	3	20	0	0	95	13	0	0
Metchnikowia reukauffii	Nectar	1051	15	10	8	0	1	0	0	11	0	0	0
Pichia kudriavzevii	Compost	1615	1	0	0	0	116	120	85	71	57	46	39
Torulaspora delbrueckii	Soil	954	20	26	7	0	51	31	0	27	0	0	0
Torulaspora delbrueckii	Beet sugar	1465	13	24	0	0	70	47	19	56	22	8	1
Citeromyces matritensis	Beet sugar	516	59	46	33	13	0	0	0	0	0	0	0
Wickerhamomyces anomalus	Beet sugar	1127	17	19	0	0	73	60	37	61	30	7	0
Wickerhamomyces anomalus	Beet sugar	1387	37	30	0	0	62	43	24	39	6	0	0
Saccharomyces cerevisiae	Bioethanol	742	15	0	0	0	102	85	81	33	0	0	0

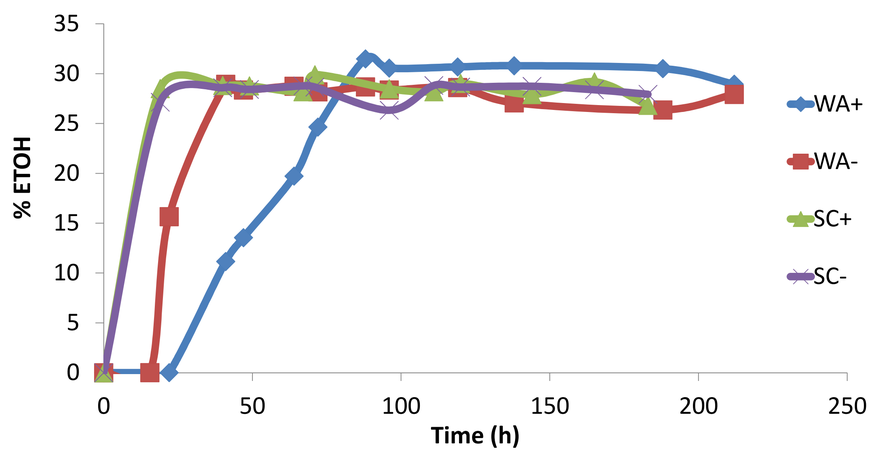

Figure 2: Ethanol yield during fermentation by *W. anomalus* and *S. cerevisiae* without (-) and with (+) lignocellulosicse related inhibitors